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Small Effect of Dopamine Release and No Effect of Dopamine Depletion on [^{18}F]Fallypride Binding in Healthy Humans

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ABSTRACT Molecular imaging has been used to estimate both drug-induced and tonic dopamine release in the striatum and most recently extrastriatal areas of healthy humans. However, to date, studies of drug-induced and tonic dopamine release have not been performed in the same subjects. This study performed positron emission tomography (PET) with [^{18}F]fallypride in healthy subjects to assess (1) the reproducibility of [^{18}F]fallypride and (2) both D-amphetamine-induced and α-methyl-*p*-tyrosine (AMPT)-induced changes in dopamin release on [^{18}F]fallypride binding in striatal and extrastriatal areas. Subjects underwent [^{18}F]fallypride PET studies at baseline and following oral D-amphetamine administration (0.5 mg/kg) and oral AMPT administration (3 g/70 kg/day over 44 h). Binding potential (BP) (BP_{ND}) of [^{18}F]fallypride was calculated in striatal and extrastriatal areas using a reference region method. Percent change in regional BP_{ND} was computed and correlated with change in cognition and mood. Test–retest variability of [^{18}F]fallypride was low in both striatal and extrastriatal regions. D-Amphetamine significantly decreased BP_{ND} by 8–14% in striatal subdivisions, caudate, putamen, substantia nigra, medial orbitofrontal cortex, and medial temporal cortex. Correlation between change in BP_{ND} and verbal fluency was seen in the thalamus and substantia nigra. In contrast, depletion of endogenous dopamine with AMPT did not effect [^{18}F]fallypride BP_{ND} in both striatum and extrastriatal regions. These findings indicate that [^{18}F]fallypride is useful for measuring amphetamine-induced dopamine release, but may be unreliable for estimating tonic dopamine levels, in striatum and extrastriatal regions of healthy humans. **Synapse 62:399–408, 2008.** Published 2008 Wiley-Liss, Inc.[†]

INTRODUCTION

Molecular imaging with single photon emission computed tomography (SPECT) or positron emission tomography (PET) can be used not only to measure D_2 receptor density, but also, under appropriate conditions, to estimate synaptic concentration of endogenous dopamine. This approach is based on the competition between certain radioligands and endogenous dopamine for D_2 receptor binding, according to pharmacological theories defined by an occupancy model (for review see Laruelle, 2000). As such, changing the concentration of dopamine will affect the number of available D_2 receptors, with increases of dopamine reducing D_2 receptor availability (i.e., specific binding) and vice versa.

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Over the past decade, SPECT or PET D_2 receptor measurement coupled with pharmacological interventions either to increase synaptic dopamine levels with a stimulant acutely or deplete dopamine levels (tonic release) rapidly, have been used to examine synaptic dopamine transmission in human brain (for review see Laruelle, 2000). However, the majority of these studies have been confined to the striatum, since the striatum is a relatively large region with an abundance of D_2 receptors (Kessler et al., 1993). Stimulating dopamine release with either intravenous or oral doses (0.3 mg/kg or 30 mg) of D-amphetamine (herein referred to as amphetamine) in healthy subjects has consistently decreased striatal binding of [^{11}C]raclopride (Boileau et al., 2006; Cardenas et al., 2004; Drevets et al., 2001; Leyton et al., 2002; Martinez et al., 2003) and [^{123}I]IBZM (Kegeles et al., 1999; Laruelle et al., 1995) by 7–18%. In contrast, depletion of cerebral dopamine with α-methyl-*p*-tyrosine (AMPT) (Engelman et al., 1968), a competitive inhibitor of tyrosine hydroxylase, increased [^{11}C]raclopride and [^{123}I]IBZM binding in striatum by 9–28% (Abi-Dargham et al., 2000; Laruelle et al., 1997; Verhoeff et al., 2001, 2002). Increase in radioligand binding was suggested to be due to removal of endogenous dopamine, subsequently unmasking D_2 receptors previously occupied by it and thus providing a measure of baseline or tonic dopamine release (Laruelle et al., 1997).

Although assessment of striatal dopamine release is important for increasing our understanding of a number of psychiatric and neurological disorders, a number of studies indicate the involvement of extrastriatal dopamine transmission in schizophrenia, addiction, neuroleptic drug interactions, and various cognitive processes (Arnsten, 1998; Laviolette and Grace, 2006; Lidow et al., 1998). Recent focus has been directed to developing high-affinity SPECT and PET radioligands that enable quantification of low-density extrastriatal dopamine receptors. [^{18}F]Fallypride is a high-affinity D_2/D_3 radioligand (K_D = ~0.2 nM) (Slifstein et al., 2004a) which, with its high specific-to-nondisplaceable binding, is capable of measuring D_2-type receptors in striatal, as well as extrastriatal regions such as thalamus, temporal cortex, substantia nigra, and limbic areas (Mukherjee et al., 2002). [^{18}F]fallypride is sensitive to changes in extracellular levels of endogenous dopamine, both in the striatum and extrastriatal regions. In nonhuman primates, a 14–49% displacement of [^{18}F]Fallypride was reported in the striatum, thalamus, amygdala, hippocampus and pituitary, following 0.6–1 mg/kg intravenous dose of amphetamine (Mukherjee et al., 1997, 2005; Slifstein et al., 2004b). Recently, amphetamine-induced displacement of [^{18}F]fallypride binding was demonstrated in both striatal and extrastriatal regions in healthy volunteers following an oral dose of 0.43 mg/kg amphetamine (Riccardi et al., 2006). Displacement of [^{18}F]fallypride was greatest in striatal subdivisions (6–11%) and substantia nigra (7%), with lesser displacement seen in amygdala, temporal cortex, and thalamus (3–4%). In addition, depletion of dopamine with AMPT was recently reported to increase [^{18}F]fallypride binding by 9–13% in striatal subdivisions and substantia nigra in a small group of healthy volunteers (Riccardi et al., 2008). To date, no study has examined the effects of drug-induced and tonic dopamine release on striatal and extrastriatal D_2 receptors within the same subjects. Although drug-induced dopamine release may be different from physiological phasic release, given the close relationship between these two modes of dopamine transmission, with tonic release modulating phasic release (Grace, 1991), we wanted to establish the effect of within-subject changes in both amphetamine-induced and tonic dopamine on [^{18}F]fallypride binding. Such within-subject examination may aid in understanding the interactions between phasic and tonic dopamine release.

The current study performed [^{18}F]fallypride PET scans at baseline and following oral amphetamine and AMPT administration in healthy human volunteers. The purpose of the study was to; (1) assess the reproducibility of measuring [^{18}F]fallypride binding (i.e., test–retest reliability), (2) for the first time, examine within the same subjects, the effects of amphetamine-induced dopamine release and AMPT-induced tonic dopamine depletion on [^{18}F]fallypride binding in both striatum and extrastriatal areas, and (3) examine the within-subject relationship between amphetamine-induced and tonic dopamine release as measured by [^{18}F]fallypride binding in both striatum and extrastriatal areas. On the basis of previous studies and the hypothesis put forth by Grace (1991), we hypothesize that amphetamine and AMPT pretreatment will decrease and increase [^{18}F]fallypride binding, respectively, and that subjects with greater amphetamine-induced release will have smaller tonic release.

METHODS

Study population

Fourteen healthy volunteers (11 male, 3 female; mean age ± SD, 29 ± 8 years; range, 20–43 years) participated in the study. All subjects were right-hand dominant and none were smokers. Subjects were free of current medical, psychiatric, and neurological illness based on history, a physical exam, routine laboratory tests (including a complete blood count, chemistries, thyroid function test, serum electrolytes, liver and kidney function, urinalysis, urine drug screen and HIV and Hepatitis B tests), and electrocardiogram. Exclusion criteria included evidence of current psychiatric or neurological condition, medi-

TABLE I. Participant demographics and clinical measures

Age (years)	29 ± 8
Education (years)	16 ± 2
Amphetamine plasma level at 3 h after administration (ng/ml)	62 ± 19.2
Amphetamine plasma level at 4.5 h after administration (ng/ml)	71.5 ± 14
AMPT plasma level (μg/ml)	20 ± 4.3
[18F]fallypride injected activity (MBq)	187 ± 11
[18F]fallypride specific activity (GBq/μmol)	70 ± 37
Interval between scan 1 and 2 (test–retest) (days)[a]	15
Interval between scan 2 (retest) and 3 (amphetamine) (days)[a]	31
Interval between scan 3 (amphetamine) and 4 (AMPT) (days)[a]	42

Values are mean ± standard deviation.
[a]Value is median.

cally significant biochemical or hematological abnormality, elevated blood pressure (>140/90), history of myocardial infarction or angina pectoris, positive urine drug screen, history of substance abuse or dependence within 6 months, body weight greater than 93 kg and pregnancy and lactation. The Radiation Safety Committee of the National Institutes of Health and the Institutional Review Board of the National Institute of Mental Health approved the study. All subjects gave written informed consent.

Radiopharmaceutical preparation

[18F]Fallypride was synthesized via nucleophilic substitution of tosyl-fallypride (ABX GmbH, Radeberg, Germany) with cyclotron-produced [18F]fluoride ion (Mukherjee et al., 1995). Preparations were conducted according to our Investigational New Drug Application no. 70,046, submitted to the US Food and Drug Administration and a copy of which is available at: http://kidb.bioc.cwru.edu/snidd/.

Scanning protocol

PET scans were acquired in three-dimensional mode using a GE Advance tomograph (GE Medical Systems, WI) and were reconstructed with the filtered-back projection algorithm which resulted in a final image resolution of 7.5 mm full width half maximum. Following the initial screening visit, subjects underwent four [18F]fallypride PET scans on four separate days in the following order – two baseline scans (test-retest) (scan 1 and 2), a scan with amphetamine administration (scan 3), and a scan with AMPT administration (scan 4). The median interval between baseline test–retest scans was 2 weeks, while there was about a 1 month interval between scan 2 (retest) and scan 3 (amphetamine) and 6 weeks between scan 3 (amphetamine) and scan 4 (AMPT) (Table I). All female subjects underwent a pregnancy test within 24 h before each PET scan. In all PET studies, an 8-min transmission scan using a ^{68}Ge rotating pin source was performed for attenuation correction. Dynamic emission scans were acquired following an intravenous bolus injection of 187 ± 11 MBq [18F]fallypride for 3 h (specific activity = 70 ± 37 GBq/μmol; mass dose/body weight = 0.017 ± 0.008 μg/kg). The initial image acquisition coincided with [18F]fallypride injection and was obtained continuously for 60 min (6 × 30 s, 3 × 1 min, 2 × 2 min, 10 × 5 min). Following this initial acquisition, two 1-h images (12 × 5 min) were acquired until about 5 h after the bolus injection. Subjects were removed from the camera for about 60 min between image acquisitions. To ensure metabolism of the radioligand was consistent among scans, subjects did not eat from 2 h before [18F]fallypride administration to the completion of the scan, and half normal saline was intravenously infused at a rate of 83 ml/h starting 1 h before [18F]fallypride injection. Subjects also consumed at least 1 l of water before and during intermissions of the PET scan. To minimize potential changes in dopamine levels, subjects were asked to refrain from consuming caffeine-containing drinks after midnight before each PET scan.

On the day of PET scan 3, a single oral dose of 0.5 mg/kg amphetamine (Dexedrine®) was administered 3 h before injection of [18F]fallypride with vital sign monitoring. Scanning was performed 3 h following amphetamine administration because there is sustained radioligand displacement following oral amphetamine (Cardenas et al., 2004). We used a slightly higher dose (0.5 mg/kg) than in previous studies (0.3–0.43 mg/kg) to induce greater dopamine release and to more easily detect changes in [18F]fallypride binding. Plasma amphetamine levels were measured at about 3 and 4.5 h following amphetamine administration to measure amphetamine levels during the PET scanning period. Plasma samples were analyzed for amphetamine using an isotope dilution procedure. The method was a minor modification of the procedure of Xie et al. (2004). The modification for plasma involved initial protein precipitation with sulfosalicylic acid. The clear supernatant was then processed as described for ultrafiltrates in Xie et al. (2004). The standard curve was linear (r = 0.999) in the range tested (1–500 ng/ml).The limit of detection was 1 ng/ml with intra and inter coefficients of variation of <5% and <7% respectively.

On the day of PET scan 4, subjects were administered 3 g/70 kg body weight AMPT (Demser®) p.o. per day over 44 h (10 doses in total) (Fujita et al., 2000; Laruelle et al., 1997). Although Laruelle et al. (1997) did not adjust AMPT dose based on body weight, they administered almost the same dose for the same length of time. Subjects with body weight more than 93 kg were excluded from the study to limit AMPT dose to 4 g/day. To prevent crystalluria during AMPT administration, subjects were asked to drink 1–2 l of water each day. In addition, half-normal saline was infused intravenously until 3 h after

the end of the fourth PET scan. A urinalysis and medical examination was conducted daily to detect crystal formation and assess tolerance to AMPT treatment. In addition, sodium bicarbonate (2 tablets of 650 mg) was given on three consecutive nights to prevent acidification of the urine during the night. To determine levels of AMPT in plasma, blood samples were collected about 90 min before, and 90 min and 3.5 h after [^{18}F]fallypride injection. Plasma levels of AMPT were determined by high-performance liquid chromatography (HPLC) using a modification of a previously reported method for the determination of plasma tryptophan and kynurenine (Hoekstra et al., 2007). In brief, 100 μl plasma samples were deproteinized with 100 μl of 0.7 M perchloric acid after addition of 50 μl of 25% ascorbic acid. Supernates obtained after centrifugation were directly injected on a 15 × 0.46 cm Microsorb C18 column eluted with 96% pH 3.7, 1.5% aqueous acetic acid and 4% methanol delivered at a flow rate of 1 ml/min, and the compounds detected fluorometrically (270/320 nm excitation/emission wavelengths). All specimens were analyzed in a single assay and the compounds determined with within-assay coefficients of variation of less than 5%.

Three subjects participated in a pilot study of only one baseline and one amphetamine scan. Of the eleven subjects who participated in the 4 scan protocol (test, retest, amphetamine, and AMPT) six subjects completed the fourth scan with AMPT while two subjects commenced the fourth scan but withdrew before completion, allowing measurement in only extrastriatal areas. Therefore, the total number of subjects was 11 for test–retest, 14 for amphetamine-induced change and 6 and 8 for AMPT-induced change in striatum and extrastriatal areas, respectively.

All subjects received a 1.5 Tesla MRI scan for coregistration and segmentation purposes. Inversion recovery fast gradient recalled-echo (IR-FGRE; TR ∼12 ms, TE ∼5 ms, flip angle 20°, voxel size: 0.86 × 0.86 × 1.2 mm), fast spin echo (FSE) T2-weighted (TR ∼3700 ms, TE ∼101 ms, flip angle 90°, voxel size: 0.43 × 0.43 × 5 mm) and fluid attenuated inversion recovery (FLAIR; TR ∼10,002 ms, TE ∼140 ms, flip angle 90°, voxel size: 0.86 × 0.86 × 5 mm) images were obtained.

Neuropsychological tests

On the morning of each scan, subjects were administered a neuropsychological battery to examine executive, attentional, processing speed, and frontostriatal processes. On amphetamine days, the commencement of the battery corresponded to 60–90 min post amphetamine administration as peak subjective and behavioral effects of oral amphetamine have shown to occur within 1–2.5 h after administration (Angrist et al., 1987; Asghar et al., 2003). For baseline 1 and amphetamine scans the battery consisted of the Symbol Digit Modality task (speed of processing), spatial span (attention and spatial working memory), Stroop color word task (response inhibition, executive function), Controlled Oral Word Association Test – CFL (verbal fluency, executive function), and the Colors Trail Test (processing speed, visual attention). For baseline 2 and AMPT scans, subjects were administered the Digit Symbol Coding test (speed of processing), Controlled Oral Word Association Test - FAS, Colors Trail Test, Letter-Number Sequencing (working memory) and the Stockings of Cambridge task (planning, frontostriatal function). To minimize practice effects, and because of the unavailability of four alternate versions of the neuropsychological tests, different tests were selected for the scans 1 and 3 vs. scans 2 and 4. Tests were administered in a fixed order with alternate forms available for some tests. Similar processes were assessed for each of the drug challenge days. The testing interval was ∼8 weeks between the baseline 1 and amphetamine sessions and ∼11 weeks between baseline 2 and AMPT sessions. One male and one female subject did not undergo neuropsychological testing following amphetamine treatment due to a previous amphetamine scan cancellation that occurred in close proximity to their retest scan. Two subjects did not complete the Letter-Number Sequencing test and two subjects were not administered the Stockings of Cambridge task due to time constraints on the testing day. Drug-induced changes in subjective mood were assessed using 10-point visual analog scales for euphoria, restlessness, anxiety, drowsiness and alertness and the Profile of Mood States (POMS; McNair et al., 1971). For amphetamine-induced emotional change, subjects rated these questionnaires pre- and about 70 min post amphetamine administration. For AMPT-induced change, subjects were administered the questionnaires at baseline (on the morning of the retest scan) and following AMPT (at the equivalent point in time on the day of the AMPT scan).

Data analysis

To correct for head movement during the scan, all [^{18}F]fallypride frames of each PET scan were realigned to a standard frame using the FLIRT algorithm (Jenkinson and Smith, 2001) for the initial image acquisition and Statistical Parametric Mapping (SPM2, The Wellcome Department of Cognitive Neurology, London, UK) for the second and third acquisitions. FLIRT was used for the initial image acquisition instead of SPM2 because for the initial set, SPM2 cut the bottom portion of images including the cerebellum. Inversion recovery MRI reoriented to the anterior commissure-posterior commissure (AC-PC) line and PET scans 2, 3, and 4 were each coregistered to

an average image of initial [^{18}F]fallypride frames from PET scan 1 using SPM2. Coregistered serial PET scans and MRI were spatially normalized to the Montreal Neurological Institute stereotaxic space using segmented gray matter images created from IR, FLAIR, and T2 MRI images in SPM2. Regions of interest defined on the caudate nucleus, putamen, thalamus, medial orbitofrontal cortex, anterior cingulate, temporal cortex, medial temporal cortex, substantia nigra, and colliculi were applied to spatially normalized PET images. Medial orbitofrontal cortex, substantia nigra, and colliculi volumes were delineated on parametric images and the location of the substantia nigra and colliculi were individually adjusted without knowing the scan identity. Striatal subdivisions were drawn on reoriented MRI and were defined according to the criteria of Mawlawi et al. (2001) for the ventral striatum, precommissural dorsal caudate, and precommissural dorsal putamen and according to Martinez et al. (2003) for the postcommissural caudate and putamen. Partial volume correction was applied to striatal subdivisions in order to recover lost spatial resolution to these regions. Partial volume correction was performed in PMOD 2.65 (pixel-wise modeling computer software; PMOD Technologies Ltd, Adliswil, Switzerland), using a model based on Giovacchini et al. (2004). Both uncorrected and corrected data from striatal subdivisions underwent region of interest (ROI) analysis.

Regional [^{18}F]fallypride BP (BP_{ND}) was calculated from 5-h data using the simplified reference tissue model (Lammertsma and Hume, 1996) implemented in PMOD by using cerebellum excluding vermis as the reference region. Test–retest variability and percent change in amphetamine and AMPT-induced BP_{ND} were calculated for each ROI. Test–retest variability was calculated as the absolute difference of baseline scan 1 (test) minus baseline scan 2 (retest) divided by the mean of test–retest and expressed as a percent. Amphetamine or AMPT-induced change in [^{18}F]fallypride BP_{ND} was determined as the percent difference in BP_{ND} between the mean baseline (scan 1 and 2) and postamphetamine (scan 3) or post-AMPT (scan 4) conditions: $\Delta BP_{ND} = ([BP_{ND\ (drug)} - BP_{ND\ (mean\ baseline)}]/BP_{ND\ (mean\ baseline)}) \times 100$. Changes in cognition from baseline to amphetamine or AMPT conditions were calculated by subtracting baseline values from drug (amphetamine or AMPT) values and dividing by baseline. Drug-induced change in mood was assessed as the difference between pre- (or baseline) and postdrug ratings on each VAS and POMS dimension.

In addition, mean parametric images of changes in [^{18}F]fallypride BP_{ND} were calculated (Gunn et al., 1997) and voxel-wise analysis of change images restricted to the entire lateral temporal cortex (i.e., small volume correction) was performed using SPM2 and SnPM, the nonparametric version of SPM. This was done because [^{18}F]fallypride showed binding in the entire temporal cortex, and changes in an area in the temporal cortex may have been overlooked by applying regions of interest.

Statistical analysis

For 11 subjects who had two baseline scans (test and retest), kinetic analysis was performed for each scan and the average values were used as baseline measurements. To determine the effects of amphetamine or AMPT on [^{18}F]fallypride BP_{ND}, repeated-measures analysis of variances (ANOVAs) with condition (baseline, amphetamine or baseline, AMPT) and region as within-subject factors, were performed using SPSS for Windows (SPSS, 1989–2004, Release 14.0). Greenhouse-Geisser corrections were used for violations of the assumption of sphericity. Separate ANOVAs were performed for striatal subdivisions and other regions (striatum and extrastriatal regions). When appropriate, paired-sample *t*-tests or Wilcoxin signed ranks tests for parametric and nonparametric data respectively, were performed to determine which regions accounted for the significant effects found on the ANOVA. Parametric and nonparametric variables were determined by the Shapiro-Wilk normality test. Right and left sided differences in regional drug-induced displacement of BP_{ND} were examined with paired *t*-tests or Wilcoxin signed rank tests. Relationships between change in BP_{ND} and change in cognition and mood, and between amphetamine and AMPT-induced change in BP_{ND}, were performed with Spearman's rank correlation. Multiple comparisons were controlled for with a false discovery rate correction (Benjamini and Hochberg, 1995).

RESULTS

[^{18}F]Fallypride uptake and BP_{ND}

[^{18}F]Fallypride was visualized and quantified in both the striatum and extrastriatal regions such as thalamus, temporal cortex, anterior cingulate, and orbitofrontal cortex (Fig. 1). BP in striatal areas were about 10–30-fold higher than values in extrastriatal areas.

Test–retest variability

Test–retest variability of [^{18}F]fallypride BP_{ND} was low for most regions, ranging from ~3.8% in the striatum, ~5% in medial and temporal cortex, to 6–8% in thalamus, medial orbitofrontal cortex, and substantia nigra. Intraclass correlation coefficient (ICC) was above 0.90 (range: 0.91–0.98) for these regions (Table II). Two regions showed test–retest variability above 10%; these were the anterior cingulate (test–retest = 21.8%, ICC = 0.54) and colliculi (test–retest = 10.9%,

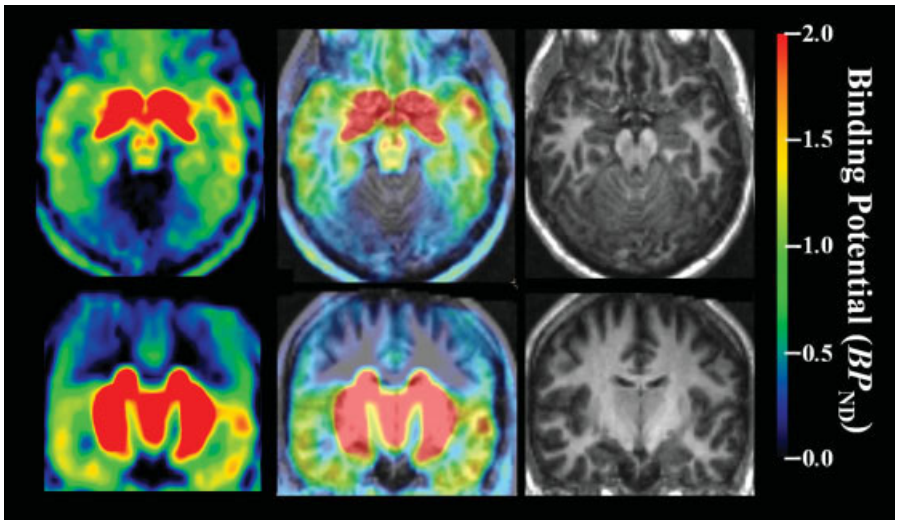

Fig. 1. Parametric image of [^{18}F]fallypride BP_{ND} in a healthy subject

TABLE II. Test-retest reproducibility of measuring [^{18}F]fallypride binding potential (BP_{ND})

Region	Test	Retest	Test–retest variability (%)	ICC
Caudate	17.1 ± 1.0	17.4 ± 1.1	3.8 ± 0.7	0.98
Putamen	19.8 ± 1.3	19.8 ± 1.4	3.7 ± 0.8	0.98
Thalamus	1.61 ± 0.08	1.68 ± 0.08	6.1 ± 1.3	0.91
Medial orbitofrontal cortex	0.60 ± 0.05	0.64 ± 0.06	6.7 ± 1.8	0.95
Anterior cingulate	0.55 ± 0.05	0.59 ± 0.05	21.8 ± 3.8	0.54
Temporal cortex	0.72 ± 0.07	0.72 ± 0.07	4.8 ± 1.2	0.98
Medial temporal cortex	0.86 ± 0.06	0.90 ± 0.07	5.1 ± 0.9	0.96
Substantia nigra	1.17 ± 0.09	1.16 ± 0.10	7.7 ± 2.0	0.95
Colliculi	1.52 ± 0.06	1.44 ± 0.08	10.9 ± 2.6	0.72

ICC, intraclass correlation coefficient.
Data are mean ± SEM.

TABLE III. Test-retest reproducibility of measuring [^{18}F]fallypride binding potential (BP_{ND}) in striatal subdivisions

Region	Test	Retest	Test-retest variability (%)	ICC
Ventral striatum	15.8 ± 1.0	16.0 ± 1.1	3.7 ± 0.6	0.98
Pre-commissural dorsal caudate	18.3 ± 2.1	17.6 ± 1.1	4.4 ± 1.2	0.96
Pre-commissural dorsal putamen	19.8 ± 1.2	18.8 ± 1.2	5.6 ± 1.0	0.96
Post-commissural caudate	13.1 ± 0.9	13.1 ± 0.9	3.0 ± 0.9	0.99
Post-commissural putamen	19.7 ± 1.2	19.2 ± 1.2	3.3 ± 0.7	0.99

ICC, intraclass correlation coefficient.
Data are mean ± SEM.

ICC = 0.72) (Table II). Given the poor reproducibility and low reliability of BP_{ND} in these regions, the anterior cingulate and colliculi were not included in further analysis of amphetamine and AMPT effects. Test–retest variability was excellent in all striatal subdivisions, ranging from 3 to 5.6% and showing ICC above 96% (Table III). Partial volume correction of striatal subdivisions resulted in poorer test–retest variability of BP_{ND} (~6%) compared with uncorrected striatal subdivisions (~4%).

Amphetamine-induced changes

A repeated-measures ANOVA using condition, region and condition by region as factors showed significant main effects for condition ($F(1,13) = 11$, $P = 0.006$), region ($F(6,78) = 335$, $P < 0.001$) and a significant condition by region interaction ($F(6,78) = 12.4$, $P = 0.003$). The effect of amphetamine on each region was further examined by paired two-tailed *t*-tests. These tests, with correction for multiple comparisons using the false discovery rate (Benjamini and Hochberg, 1995), revealed significant amphetamine-induced decreases in [^{18}F]fallypride BP_{ND} in, by rank order, the substantia nigra (13.1%), medial orbitofrontal cortex (13%), putamen (12.3%), medial temporal cortex (7.8%), and caudate (7.6%) (Table IV). Lower levels of displacement were seen in the thalamus and temporal cortex (7%) but these did not reach significance. For striatal subdivisions, a repeated-

TABLE IV. Amphetamine-induced changes in [^{18}F]fallypride binding

Region	Baseline BP_{ND}	Post-Amphet BP_{ND}	% Change
Caudate	17.7 ± 0.9	16.3 ± 0.9	−7.6 ± 2.7*
Putamen	20.4 ± 1.1	17.9 ± 1.1	−12.3 ± 2.8*
Thalamus	1.71 ± 0.07	1.60 ± 0.10	−7.0 ± 3.0
Medical orbitofrontal cortex	0.65 ± 0.05	0.56 ± 0.05	−13.0 ± 4.8*
Temporal cortex	0.79 ± 0.07	0.73 ± 0.07	−7.0 ± 3.1
Medical temporal cortex	0.94 ± 0.06	0.87 ± 0.06	−7.8 ± 2.8*
Substantia nigra	1.23 ± 0.08	1.07 ± 0.08	−13.1 ± 3.1*

Data are mean ± SEM.
*Significant change with correction for multiple comparisons using the false discovery rate.

TABLE V. Amphetamine-induced changes in [^{18}F]fallypride binding in striatal subdivisions

Region	Baseline BP_{ND}	Post-Amphet BP_{ND}	% Change
Ventral striatum	16.1 ± 0.8	14.7 ± 0.8	−8.5 ± 2.8*
Pre-commissural dorsal caudate	18.5 ± 0.9	16.4 ± 0.9	−11.5 ± 2.7*
Pre-commissural dorsal putamen	20.0 ± 1.0	17.2 ± 1.0	−13.5 ± 2.6*
Post-commissural caudate	13.6 ± 0.8	12.6 ± 0.9	−7.9 ± 3.0*
Post-commissural putamen	20.1 ± 1.0	17.4 ± 1.1	−13.6 ± 3.0*

Data are mean ± SEM.
*Significant change with correction for multiple comparisons using the false discovery rate.

measures ANOVA similarly revealed significant main effects for condition ($F(1,13) = 14.2$, $P = 0.002$), region ($F(4,52) = 76.9$, $P < 0.001$) and a significant condition by subdivision interaction ($F(4,52) = 21.2$, $P < 0.001$). Paired-sample *t*-tests showed significant amphetamine-induced displacement in all subdivisions (Table V), ranging between 8 and 14%. Partial volume corrected data showed almost identical statistical results. Right–left differences in amphetamine-induced displacement was found in the putamen only ($P = 0.007$) with the right side showing greater displacement (19.5%) compared with the left side (12.1%). Analysis of striatal subdivisions revealed that the left precommissural dorsal putamen showed greater displacement (15.4%) than the right side (11.8%), while the right postcommissural caudate had greater displacement (10.3%) than the left side (5.6%).

Correlations between amphetamine-induced dopamine release and changes in cognition and mood revealed significant negative correlations between changes in the Controlled Oral Word Association Test (CFL) and changes in BP_{ND} in the thalamus ($\rho = -0.9$, $P < 0.001$, corrected for multiple comparisons) and the substantia nigra ($\rho = -0.9$, $P < 0.006$, corrected for multiple comparisons), with greater amphetamine-induced dopamine release being associated with better cognitive performance. No significant correlations were found for other cognitive and mood measures and in other regions. Voxel-wise analysis with SPM and SnPM did not detect correlations between amphetamine-induced dopamine release and cognitive changes in subregions of the lateral temporal cortex.

Plasma amphetamine levels were 62 ± 19.2 ng/ml at ~3-h post administration and 71.5 ± 14 ng/ml at ~4.5 h (Table I).

AMPT-induced changes

Repeated-measures ANOVAs in striatal subdivisions and other regions revealed no overall effect of condition or interaction of condition by region. Paired-sample *t*-tests or Wilcoxin signed ranks tests (two-tailed) confirmed that there were no significant changes in BP_{ND} following AMPT treatment in striatal and extrastriatal regions. There was large inter-subject variability in AMPT-induced changes in BP_{ND}, with some subjects showing large decreases in binding. Percent change of BP_{ND} ranged between −3.8 and 1.1% in striatal and −11.7 to 1.2% in extrastriatal regions. No right/left-sided differences in AMPT-induced change in BP_{ND} were observed. Plasma levels of AMPT remained stable over the course of the PET scan. The average plasma AMPT concentration was 20 ± 4.3 μg/ml (range 14–27 μg/ml). Plasma levels of AMPT did not correlate with AMPT-induced change in BP_{ND} in any region.

Correlation between amphetamine and AMPT-induced changes

There were no significant correlations between amphetamine and AMPT-induced changes in BP_{ND} in striatal or extrastriatal regions.

DISCUSSION

We examined the test–retest variability of [^{18}F]fallypride and the effects of changes in amphetamine-induced and tonic (with AMPT) dopamine and their relationship on [^{18}F]fallypride binding in both striatal and extrastriatal regions. This study showed that the reproducibility of [^{18}F]fallypride measurement in striatal and extrastriatal regions was excellent. With the exception of the anterior cingulate and colliculi, the average absolute difference in

[^{18}F]fallypride BP_{ND} between test and retest was under 10% for all regions.

Consistent with Riccardi et al. (2006), we found that oral administration of amphetamine 3 h before [^{18}F]fallypride injection significantly reduced radioligand binding to D_2 receptors in both the striatum and extrastriatal regions, with the exception of the thalamus and temporal cortex. Amphetamine-induced release ranged between 7 and 14%, with the greatest displacement occurring in the pre- and postcommissural putamen, substantia nigra and medial orbitofrontal cortex, and smaller displacement in the caudate, ventral striatum, temporal cortex, and thalamus. As expected, the magnitude of this displacement was slightly higher than that reported by Riccardi et al. (2006) using [^{18}F]fallypride and a slightly lower dose (0.43 mg/kg) of oral amphetamine. Dopamine release in the thalamus and temporal cortex (both 7%) was reasonably greater than the 3–4% displacement observed by Riccardi et al. Nevertheless, these displacements did not remain significant after controlling for the false discovery rate, presumably because of intersubject variability. Further, our magnitude of regional percent change exceeds the test–retest variability of [^{18}F]fallypride measurement, indicating that such displacement was not due to poor [^{18}F]fallypride reproducibility.

Although the degree of amphetamine-induced dopamine release was slightly higher than previously reported with [^{18}F]fallypride, the rank order of regional displacement was similar. As in the study by Riccardi et al. (2006), the substantia nigra showed a high level of displacement (13%), which was comparable to that seen in the putamen. Although this is speculative, as discussed by Riccardi et al., such unexpectedly high [^{18}F]fallypride displacement in the substantia nigra may be related to the different proportion of D_2 receptors in the substantia nigra and striatum configured in the high- and low-affinity agonist states with a greater proportion of high-affinity state in the former. Another possible explanation is regional differences in the proportion of D_2 and D_3 receptors (Murray et al., 1994) although there has not been a report that fallypride binds preferentially to D_3 receptors, which exists with high density in substantia nigra.

With the exception of the ventral striatum, the magnitude of [^{18}F]fallypride displacement in striatal subdivisions was similar to the 8–16% displacement of [^{11}C]raclopride following an intravenous dose of 0.3 mg/kg amphetamine (Drevets et al., 2001; Martinez et al., 2003). This similarity demonstrates that a relatively high dose of oral amphetamine (0.5 mg/kg) is just as effective as an intravenous dose in displacing radioligand binding, and further, that a high-affinity D_2 radioligand such as [^{18}F]fallypride, has a comparable sensitivity to competition from changes in amphetamine-induced dopamine release as does [^{11}C]raclopride. There are several benefits to administering amphetamine orally rather than intravenously. Most importantly, the oral route should result in fewer side effects, which is critical for subject retention and safety. In addition, the duration between oral administration and radioligand injection allows assessment of neuropsychological processes for determination of possible relationships between regional amphetamine-induced dopamine release and changes in cognition and mood.

In this study, we also examined the relationship between changes in dopamine and cognitive function as previous studies have yielded promising findings (for a review see Cropley et al., 2006), including the study by Riccardi et al. (2006) using [^{18}F]fallypride. Despite examining similar cognitive functions as did Riccardi et al. (2006), we did not replicate their finding of significant correlations between amphetamine-induced dopamine release and change in measures of attention and speed of cognitive processing. However, we did observe significant positive correlations (corrected for multiple measures and regions with the false discovery rate (Benjamini and Hochberg, 1995), between amphetamine-induced dopamine release in the thalamus and substantia nigra and change in the controlled oral word association test, a test of phonological verbal fluency and executive function. Verbal fluency involves activation of prefrontal, left temporal, anterior cingulate as well as thalamic regions (Frith et al., 1995) and is associated with dopamine function in striatum and frontal cortex (Lawrence et al., 1998; Rinne et al., 2000). As the thalamus forms part of the fronto-striato-thalamic neuronal circuitry (Alexander and Crutcher, 1990), such a relationship with thalamic dopamine release is plausible, whereas in the substantia nigra the relationship may reflect overall dopamine release. Nevertheless, these regional correlations should be interpreted with caution as the influence of practice on cognitive change cannot be determined. The lack of correlations between regional dopamine release and other cognitive measures may be related to insufficient power due to the small sample size and intersubject variability in amphetamine-induced dopamine release and cognitive change.

Although the sample size was small ($n = 6$), in contrast to Riccardi et al. (2008), we found no effect of AMPT-induced dopamine depletion on [^{18}F]fallypride binding in both striatal and extrastriatal regions in our sample of healthy volunteers. In the Riccardi et al. study, 71.4 mg/kg AMPT over 26 h resulted in significantly increased [^{18}F]fallypride binding (9–13%) in the caudate, putamen, ventral striatum and substantia nigra. In comparison, the effect of a slightly lower dose of AMPT on [^{18}F]fallypride binding in the current study was variable, showing trends of paradoxical decreases in binding or no change, and large

intersubject variability. Movement of head was not a cause of the paradoxical decreases because no subject showed significant akathisia, and the PET images were corrected for movement. Our findings also differ from those of previous studies reporting AMPT-induced increases of D_2 radioligand binding in the striatum using [^{123}I]IBZM (+28%) (Laruelle et al., 1997) and [^{11}C]raclopride (+13 to 18.5%) (Verhoeff et al., 2001, 2002), and in the temporal cortex with [^{123}I]epidepride (+13%) (Fujita et al., 2000).

The reasons for this discrepancy between our results and those from prior studies are unclear but are probably not related to the dose of AMPT. For example, the resulting steady-state levels of AMPT in plasma in our study (20 ± 4.3 μg/ml) were similar to those of Laruelle et al. (1997) and Fujita et al. (2000). On the other hand, although plasma AMPT levels in the current study were comparable with those in previous studies, our subjects showed relatively small subjective and objective AMPT effects, at least in comparison to a previous AMPT study carried out by this same group (Fujita et al., 2000). In that study, subjects experienced strong AMPT effects, indicated by two withdrawals before radioligand infusion due to akathisia and anxiety, and a greater necessity for treatment of side effects. In comparison, none of the eight subjects in the current study withdrew before starting the PET scan. This apparently weaker AMPT effect in our subjects may have been a result of insufficient central dopamine depletion, despite comparable peripheral AMPT plasma levels to other studies.

A main difference between the current study and previous AMPT radioligand binding studies was that we administered a single oral dose of amphetamine before AMPT administration. Whether this had any effect on subsequent D_2 receptor measurement or on the integrity of the dopamine system is unclear. As the elimination half-life of amphetamine in adults is ~13–14 h (Martinsson et al., 2003), there would be no residual amphetamine 1 week after its administration. The shortest interval between amphetamine and AMPT scans was 2 weeks. However, another possibility is that amphetamine exposure altered the sensitivity of the dopamine system, as was recently shown in a study of healthy males (Boileau et al., 2006). Specifically, that study reported increased dopamine release in the striatum 2 weeks and 1 year following three single doses of oral amphetamine, relative to the initial amphetamine dose (i.e., sensitization).

One limitation in the current and previous studies using [^{18}F]fallypride (Riccardi et al., 2006, 2008) is using cerebellum as the reference region. If specific binding exists in cerebellum that is affected by dopamine levels as shown for [^{11}C]FLB 457 (Asselin et al., 2007; Montgomery et al., 2007), drug-induced changes in [^{18}F]fallypride binding would have been underestimated. However, changes of [^{18}F]fallypride binding in cerebellum were not detected in monkey with amphetamine administration (Slifstein et al., 2004b). Therefore, using a reference tissue model in the fallypride studies is unlikely to have caused underestimation in drug-induced changes. Another limitation is that the four PET, test, retest, amphetamine, and AMPT scans were performed in this fixed order. Although it is preferable to apply a randomized design, particularly to study neuropsychological effects, we performed scans in the fixed order because only limited information was available on prolonged effects of amphetamine and AMPT administration and a primary goal of the current study was to examine whether dopamine levels affect [^{18}F]fallypride binding. The length of residual effects of amphetamine and AMPT must be carefully studied before applying a randomized design.

In summary, the current study demonstrates good reproducibility of [^{18}F]fallypride measurements, and confirms the feasibility of measuring amphetamine-induced dopamine release in striatal and most extrastriatal regions using oral amphetamine in healthy subjects. However, contrary to recent observations, our results suggest that [^{18}F]fallypride with AMPT treatment may be unreliable for estimating tonic or baseline dopamine levels in humans.

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